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The effect of salt concentration on the electrophoretic speed of a polyelectrolyte through a nanopore

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In a previous paper [1] a hydrodynamic model for determining the electrophoretic speed of a polyelectrolyte through an axially symmetric slowly varying nanopore was presented in the limit of a vanishingly small Debye length. Here the case of a finite Debye layer thickness is considered while restricting the pore geometry to that of a cylinder of length much larger than the diameter. Further, the possibility of a uniform surface charge on the walls of the nanopore is taken into account. It is thereby shown that for fixed ζ-potentials on the surface of the polyelectrolyte and on the pore wall, the electrophoretic speed is independent of the Debye length. The translocation speed depends on the salt concentration only to the extent that the ζ-potentials depend on it, and further, this dependence is very weak. It is shown that the calculated transit times are consistent with recent measurements in silicon nanopores that reveal this insensitivity to salt concentration.

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The translocation of polymers across nanometer scale apertures in cell membranes is a common phenomenon in biological systems [2]. If the polymer carries a charge, an applied electric potential can drive the translocation. The change in electrical conductance of a single nanopore as a polymer transits the pore can be reliably detected and used to characterize the polymer [3]. A number of experimental studies [4, 5, 6, 7] as well as a few theoretical ones [1, 8] on the electrically driven translocation of polymers across nanopores have appeared recently. Interest in the phenomenon is to a large extent motivated by the possibility of refining it to the point where the base sequence of a DNA strand can be read with single base resolution as the DNA transits the pore [9]. This would provide a sequencing method that is faster and cheaper than existing ones by many orders of magnitude. A technological challenge is the trade off between noise and resolution. In typical experiments with solid state nanopores a single base pair transits the pore in about $\sim 10^{-8}$ sec – much too short to be resolved. On the other hand the voltage across the pore cannot be sufficiently reduced to slow down the DNA because then the change in current would not be detectable above the noise. A theoretical analysis of the problem to determine how the translocation speed depends on the controllable parameters is therefore of value in guiding the experimental work.

In an earlier paper [1] (henceforth Paper A) a hydrodynamic model was proposed for describing the process of electrically driven translocation across the nanopore. The speed of translocation is determined by a balance of electrical and viscous forces arising from within the pore with proper accounting for the co- and counter-ions in the electrolyte. The underlying physics is not unlike that of electrophoresis of small charged particles in an applied electric field except that here the proximity of the pore walls play an important role. The translocation speed was explicitly calculated for cylindrically symmetric pores by assuming an infinitely thin Debye layer and slowly varying pore radius. The calculated translocation speed was shown to be in close agreement with experimental measurements [7] in solid state nanopores. The assumption of infinitely thin Debye layers was justified because of the high concentration of salt (1 M KCl) in the electrolyte used in the experimental work. More recently Smeets *et al.* [10] have published experimental data on a solid state nanopore for an electrolyte with KCl concentration varying from 50 mM to 1.0M. Remarkably, it was found that the most probable translocation time either did not vary at all with salt concentration or the variation was too small to be detected. In this paper the translocation speed is calculated based on the mechanism proposed in Paper A but allowing for a finite Debye Layer thickness while restricting the geometry to a long cylindrical pore. The objective is to determine whether the proposed hydrodynamic model is consistent with the observed experimental dependance of the translocation speed on salt concentration.

Figure 1 shows the geometry for our simplified calculation. The pore shape in the experiment actually resembles a hyperboloid with a smallest diameter of 10.2 nm. Smeets *et al.* [10] report that the bulk conductance of the pore is equivalent to that of a cylindrical nanopore of identical diameter and length $L = 34$ nm. For the purpose of comparing our calculation with experiments, we will consider a cylindrical pore with these dimensions. Moreover, we will assume the flow field to be uniform in the axial direction, an assumption that is strictly valid only for an infinitely long cylinder. Let us model the part of the polyelectrolyte inside the pore by a straight rigid cylindrical rod (of radius $a = 1$ nm) that is co-axial with the cylindrical pore (of radius $R = 5.1$ nm) and translocating at a velocity v in the axial direction (x). Such a model is reasonable since the persistence length of double

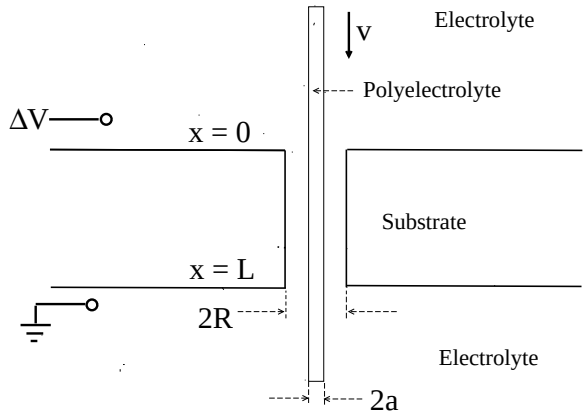

FIG. 1: Geometry of the pore region.

stranded DNA (ds-DNA) is about 50 nm. In the absence of an applied pressure gradient (the inlet and outlet reservoirs are both at atmospheric pressure), the flow velocity $\mathbf{u} = \hat{\mathbf{x}}\, u(r)$ in the region between the polyelectrolyte and the pore wall satisfies Stokes equation (the Reynolds number Re $\sim 10^{-4}$):

$$\mu \nabla^2 \mathbf{u} + \hat{\mathbf{x}} \rho_e(r)\ E_0 = 0 \tag{1}$$

where μ is the dynamic viscosity of the electrolyte, E_0 is the applied electric field in the pore and $\rho_e(r)$ is the electric charge distribution due to the co and counterions as a function of the distance from the axis (r). On account of axial symmetry of the cylindrical geometry considered, the movement of ions due to the current or bulk motion does not change the electric charge density in the diffuse layer. Thus, $\rho_e(r)$ is both the equilibrium charge density ($E_0 = v = 0$) as well as the charge density after a potential difference is applied across the cylinder (E_0 and $v \neq 0$). Poisson's law relates ρ_e to the electric potential ϕ:

$$\epsilon \nabla^2 \phi = -\rho_e \tag{2}$$

where ϵ is the dielectric constant of the electrolyte. Equations (1) and (2) must be solved with boundary conditions

$$\phi(r = a) \ = \ \zeta_p \tag{3}$$
$$\phi(r = R) \ = \ \zeta_w \tag{4}$$

where ζ_p and ζ_w are the ζ-potentials at the surface of the polyelectrolyte and the wall respectively. The classical condition of no-slip at the walls imply that

$$u(r = a) \ = \ v \tag{5}$$
$$u(r = R) \ = \ 0. \tag{6}$$

Equations (1) and (2) imply that the function $f = u - \epsilon E_0 \phi/\mu$ satisfies the equation

$$\nabla^2 f = \frac{1}{r}\frac{d}{dr}\left(r \frac{df}{dr}\right) = 0 \tag{7}$$

with boundary conditions

$$f(a) = v - \frac{\epsilon E_0 \zeta_p}{\mu} \tag{8}$$
$$f(R) = -\frac{\epsilon E_0 \zeta_w}{\mu}. \tag{9}$$

The equation for f is readily integrated, giving us the flow profile $u(r)$:

$$\frac{u(r)}{u_e} = \frac{\phi - \zeta_w}{\zeta_p} + \left(\frac{v}{u_e} + \frac{\zeta_w - \zeta_p}{\zeta_p}\right)\frac{\ln(r/R)}{\ln(a/R)} \tag{10}$$

where $u_e = \epsilon E_0 \zeta_p/\mu$ is a characteristic electrophoretic velocity. The potential ϕ is determined from the Poisson-Boltzmann equation which is obtained on substituting the Boltzmann distribution on the right hand side of equation (2):

$$\epsilon \nabla^2 \phi = -\sum_k e z_k n_k^{(\infty)} \exp\left(-\frac{z_k e \phi}{k_B T}\right). \tag{11}$$

Here z_k is the valence and n_k^∞ the far field concentration of ion species k, e is the magnitude of the electronic charge, k_B is the Boltzmann constant and T the absolute temperature of the electrolyte. However, for the purpose of determining the translocation speed v, an explicit solution for ϕ will not be needed. The required velocity is obtained from the condition that the total force on the section of the polymer inside the pore is zero:

$$F_e + F_v = 0 \tag{12}$$

where F_e and F_v are the electric and viscous forces per unit length of the polymer. If λ is the charge per unit length of the polymer then

$$F_e = \lambda E_0 = -2\pi a \epsilon E_0 \phi'(a) \tag{13}$$

by Gauss's law. The viscous force, $F_v = 2\pi a \mu u'(a)$ can be calculated using (10):

$$\frac{F_v}{2\pi\mu u_e} = a\frac{\phi'(a)}{\zeta_p} + \left(\frac{v}{u_e} + \frac{\zeta_w - \zeta_p}{\zeta_p}\right)\frac{1}{\ln(a/R)}. \tag{14}$$

On substituting equations (13) and (14) into (12) we get

$$v = u_e\left(1 - \frac{\zeta_w}{\zeta_p}\right) = \frac{\epsilon E_0 \zeta_p}{\mu} - \frac{\epsilon E_0 \zeta_w}{\mu}. \tag{15}$$

Equation (15) for the translocation speed is the main result of this paper. Surprisingly, equation (15) is identical to the result we would have obtained if we had made the assumption of infinitely thin Debye layers as we did in Paper A. To see this, observe that equation (15) together with the Helmholtz-Smoluchowski slip boundary condition would imply a uniform flow $u(r) = -\epsilon\zeta_w E_0/\mu$ in the fluid which would satisfy the condition of zero force on the polyelectrolyte considered together with its Debye layer. Equation (15) also follows directly (with $\zeta_w = 0$)

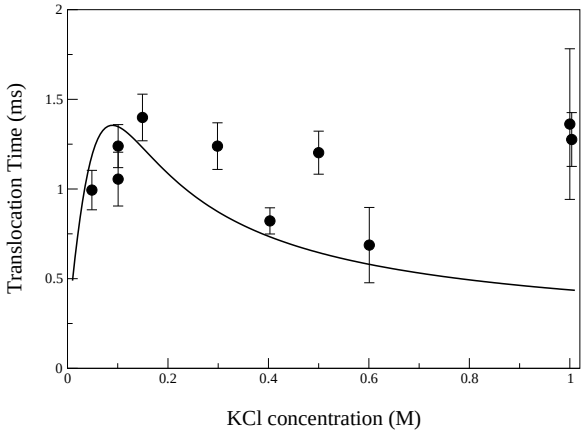

FIG. 2: Translocation times for 16.5 μm long ds-DNA through a 10.2 nm diameter solid state nanopore. Solid line is calculated from equations (15), (16) and (17), the symbols are replotted from the data presented in Figure 4(b) (inset) of Smeets *et al.* [10]

from equation (12) for the translocation speed in Paper A if one assumes a uniform cylinder for the pore shape. In addition, it has the following very simple interpretation in the limit of thin Debye layers: the part $-\epsilon E_0 \zeta_w/\mu$ is the electro-osmotic flow through the pore generated by the applied field and $\epsilon E_0 \zeta_p/\mu$ is simply the electrophoretic speed of an object of arbitrary shape in a reference frame fixed to the moving fluid in the nanopore [**?**].

Equation (15) will now be compared to experimental data due to Smeets *et al.* referenced earlier [10]. In the Debye-Huckel approximation the ζ potential of the polyelectrolyte, ζ_p is related to its linear charge density λ through the formula (see Paper A)

$$\zeta_p = \frac{\lambda \lambda_D}{2\pi a \epsilon} \frac{K_0(a/\lambda_D)}{K_1(a/\lambda_D)}, \tag{16}$$

where λ_D is the Debye length and K_n are the modified Bessel functions of order n. For a univalent salt like KCl the Debye length (in nm) is given by [11] $\lambda_D = 0.303/\sqrt{c}$ where c is the Molar concentration of the salt. The experimental data is in the range 0.05 to 1.0 M so that λ_D ranges from 0.30 nm to 1.36 nm. Since $R = 5.1$ nm and $a = 1.0$ nm, there is no significant overlap between the Debye layers at the polyelectrolyte and the wall for concentrations above 0.05 M, though for even smaller concentrations such effects may be expected. Thus, it is reasonable to use the expression (16) which is strictly true only for an isolated infinite rigid rod in an unbounded electrolyte. The dielectric constant $\epsilon/\epsilon_0 = 80$ and dynamic viscosity $\mu = 8.91 \times 10^{-4}$ Pa s for the electrolyte are taken as those of water. For the linear charge density on the DNA we take 5.9 electronic charges per nm reduced by the Manning factor of 4.2, thus $\lambda = -2.25 \times 10^{-10}$ C/m. This assumption is supported by recent force measurement experiments [12] that show that polyelectrolyte charge is reduced by the classical Manning factor when the DNA is inside the pore over a wide range of salt concentrations. The electric field intensity is obtained by assuming that the entire voltage drop of 120 mV occurs over the length of the equivalent cylinder which is $L = 34$ nm, thus, $E_0 = -3.53 \times 10^6$ V/m. The ζ-potential at the SiO_2 wall may be obtained from the expression

$$\zeta_w = a_0 - a_1 \log_{10} c \tag{17}$$

where c is the molar concentration of K^+ ions. The functional form of the dependence on concentration follows in the low counter-ion concentration limit from the nonlinear Gouy-Chapman model of the Debye layer in case of symmetric electrolytes. However, it has been shown to provide a good empirical fit to experimental data for counter-ion concentrations up to 1.0M [13]. For KCl on silica $a_0 \approx 0$ and $a_1 \approx -30$ mV.

The translocation velocity, v is calculated from equation (15) for a range of concentrations from 0.01 to 1.01M. The corresponding translocation time for a $L_p = 16.5\ \mu m$ long DNA, $t = L_p/v$ is shown as the solid line in Figure 2. A notable feature is the lack of sensitivity of the translocation time to the salt concentration: it changes by at most a factor of three when the salt concentration ranges over two orders of magnitude. Taking into account the considerable scatter in the experimental data and the various approximations made in the theory, the agreement between the two is quite reasonable, pointing to the adequacy of the underlying hydrodynamic model. The existence of a maximum in the translocation time at a concentration of about 0.1 M KCl seems to be supported by the data, although one cannot be completely certain of this on account of the uncertainty in the data. The principal uncertainties involved in applying the hydrodynamic model to nanopores were discussed in Paper A. Those same considerations apply to the current calculations as well and need not be repeated here. It should also be kept in mind that although the motion of the polymer is treated as a unidirectional translation at constant speed, the actual translocation takes place via a drift diffusion process as described by Lubensky and Nelson [8]. Here it is assumed, as is done in the classical theory of Brownian motion of particles, that, the mean part of the motion of the polymer may be obtained through the solution of a classical hydrodynamics problem that ignores the fluctuating forces. The hydrodynamic model or indeed any model that localizes the entire resistive force at the pore region would predict a translocation speed that is independent of polymer length. This is valid only for polymers that are not too long (see Paper A). For very long polymers the resistive force has an entropic part as discussed by various authors [14, 15, 16]. Störm *et al.* [17] have suggested that the viscous drag on the randomly coiled part of the polymer lying outside the pore could also be significant.

DNA translocation experiments that have been performed to date can be divided into two classes; those that use a natural protein nanopore (α-hemolysin) on a

lipid membrane [3, 4], and those that use a mechanical nanopore on a solid substrate made by specialized techniques [6, 18]. Although the principle is similar, these two types of nanopores differ with respect to some important details. One essential physical difference is that the narrowest part of the α-hemolysin pore is about 1.5-2.0 nm in diameter so that only single stranded DNA or RNA is able to pass through it. When the pore is blockaded by such a single strand the blockade is almost complete in that very few ions and probably none of the water is able to pass through the blocked pore. Although solid state pores can be made with pore sizes approaching 1 nm, most of the experiments to date have been done with $5 - 10$ nm diameter pores which can be made in a more reliable and reproducible manner. These larger diameter pores admit both single and double stranded DNA, and furthermore dsDNA can enter the pore in a folded fashion, notwithstanding the relative rigidity of these polymers [7]. The main observable difference in terms of translocations across the two kinds of pores is that the polymer passes through the solid state pores about two orders of magnitude faster than it does through α-hemolysin pores. It is important to stress that the analysis presented here applies to only the $5 - 10$ nm solid state nanopores. Although a similar hydrodynamic model could be constructed to model the viscous force arising out of the water in the vestibular part of the α-hemolysin pore, such a model must of necessity differ from the current one in the details of its formulation. Furthermore, the applicability of the continuum equations for electrostatics and hydrodynamics would be questionable to a much greater degree than in the analysis presented in this paper. It has been suggested that in order to explain the much slower translocation speed in protein pores, something other than hydrodynamics is needed: perhaps an atomic level pore-polymer interaction, an electrostatic self-energy barrier [19] or the energy cost associated with stripping hydration layers from the polymer as it enters the pore. The results derived in this paper neither supports nor refutes the validity of these alternate mechanisms for the $1.5 - 2.0$ nm protein pores. It does however show that for the $5 - 10$ nm solid state pores hydrodynamic resistance can explain the experimental data in the absence of any of the other mechanisms.

In conclusion, the hydrodynamic model introduced in Paper A to calculate the average transition time of a polyelectrolyte across a nanopore under an applied electric field was extended to treat the case of a finite Debye layer thickness, though the geometry was restricted to the simple case of a cylindrical pore. The predicted translocation times are found to be consistent with available experimental data to within the uncertainties inherent in the experiment and the theory. As a final remark, it is worth noting a few practical implications of the simple model presented here in relation to the problem of how one needs to tune the available parameters to make the translocation time as large as possible. First, Figure 2 shows that an optimal salt concentration exists for which the translocation speed is a maximum, though the gain here is no more than a factor of 3. A better strategy is suggested by equation (15) which shows that v vanishes if $\zeta_w = \zeta_p$. Physically this essentially amounts to balancing the electrophoretic migration of the DNA against an opposing electroosmotic flow generated at the wall. In principle this could be achieved by using an alternate substrate, a coating on the existing substrate or a physical or chemical treatment of it that alters its ζ-potential. The object is to select a substrate such that $\zeta_w \approx \zeta_p$ and then "fine tune" the salt concentration to achieve a closer match. As an example, Poly(methyl methacrylate) (PMMA) is a commonly used substrate in microfluidic application for which $a_0 = -4.06$ mV and $a_1 = -12.57$ mV [20]. Using these values in (17) and plotting the result together with equation (16) it is easily seen that the two curves intersect at a salt concentration of about 0.6 M. Operating near this molarity with a PMMA substrate should result in significantly slower translocations.

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